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Humoral immune response of buffalo heifers (*Bubalus bubalis*) vaccinated with B19 strain of *Brucella abortus*
Resposta imune humoral de bubalinos (*Bubalus bubalis*) vacinados pela cepa B19 de *Brucella abortus*

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RESUMO: A brucelose se encontra distribuída mundialmente. No Brasil apresenta maior prevalência para a espécie *Brucella abortus* que apesar de ser endêmica, encontra-se distribuída de forma heterogênea entre as diferentes regiões do Brasil. O presente estudo teve por objetivo avaliar a resposta imune humoral de bezerras bubalinas criadas na Mesorregião do Médio Amazonas, vacinadas com a cepa B19 de *B. abortus* na idade preconizada pelo Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose (PNCEBT). Os animais selecionados foram 36 fêmeas (grupo teste) e 6 machos (grupo controle) com idades entre 3-8 meses. No dia 0 foi realizada a colheita de sangue em todos os animais, em seguida as fêmeas receberam vacina comercial com amostra B19 em dose padrão, com posteriores coletas de sangue até aos 390 dias. Os soros sanguíneos foram avaliados nas provas de soro aglutinação: Antígeno Acidificado Tamponado - AAT e 2- Mercaptoetanol - 2ME. Os resultados para todas as amostras coletas no dia 0 apresentaram resultado negativo na reação. Aos 30 e 60 dias todas as fêmeas apresentaram 100% de reação na prova AAT e titulação de 200, iniciando o declínio da reação aos 90 dias nas duas provas. Durante o estudo um animal se mante reativo nas duas provas até 360 dias e, somente aos 390 dias 100% das fêmeas obtiveram reação negativa nas duas provas, durante todo o estudo os machos foram não reativos. A vacinação mostrou-se eficaz para a imunização, sendo uma ferramenta importante na profilaxia da brucelose na espécie estudada, bem como as provas aplicadas para o diagnostico dessa enfermidade em búfalos a nível regional.
Palavras-chave: brucelose, bubalinos, vacina B19, soro aglutinação, resposta imune.

ABSTRACT: Brucellosis is a worldwide distributed disease. In Brazil it is unevenly distributed among the different country regions, being endemic and the highest prevalence is for *Brucella abortus* species. The present study aimed to evaluate the humoral immune response of buffalo calves raised in the Middle Amazon Mesoregion, northern Brazil, vaccinated with B19 strain of *B. abortus* at the age set by the National Program for the Control and Eradication of Brucellosis and Tuberculosis (PNCEBT). The selected animals were 36 females (test group) and 6 males (control group) aged 3-8 months. On day 0 blood sampling was performed in all animals then females received commercial vaccine with standard-dose B19 sample, with subsequent blood collections throughout 390 days. The serum samples were evaluated through serum agglutination tests: Modified Rose Bengal Plate Test (MRBPT) titration 200 and 2 - Mercaptoethanol - 2ME. The results for all samples collected on day 0 were negative in reaction, however at 30 and 60 days all females showed 100% test reaction and titration MRBPT 200, with the decline beginning of the reaction

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occurring at 90 days in the both tests, although during the study one animal showed reactive in both tests up to 360 days, at 390 days 100 % of the females and males had a negative reaction in both tests throughout the study. that being do, it can be concluded that commercial vaccination with standard-dose B19 sample was effective for immunization, which can be considered as an important tool in the prophylaxis of brucellosis in the studied species, as well as the tests accuracy applied to the diagnosis of this disease in buffaloes at regional level .
Key Words: brucellosis, buffalo, B19 strain, serum agglutination test, immune response

INTRODUCTION

The world's livestock continues to suffer economic losses due to brucellosis dissemination in developing countries which in cattle and buffaloes producing abortion, retained placenta, and increase the calving interval (PAULIN, 2003; FERREIRA NETO, 2009), thus causing low reproductive efficiency and falling production of meat and milk in cattle and buffalo herds (PAULIN & FERREIRA NETO, 2008).

The domestic buffalo has been introduced for over a century throughout the Brazilian Amazon, with the introduction of animals of Mediterranean breed and Swamp type in Marajo Island, with further importation of Murrah and Jafarabadi breeds from India (ROSE et al. 2007).

Actually, buffalo species is spread over large areas of the Amazon basin floodplain and Marajo Island (SOUZA et al. 2002), showing an annual growth of over 12 % which is five times more than the growth of cattle (ROSE et al. 2007), but has received a very low technological input in despite being exploited for meat, milk, animal draft production (LOURENÇO JUNIOR & GARCIA, 2008). As the buffalo production system is more widespread in the northern part of the country, where Pará state owns 65% of the national herd (PAULIN et al. 2006) this species has been also affected by the disease, however few studies are directed to the buffalo.

Actually, Brazil has established a task force in order to set up a national control brucellosis and tuberculosis named National Program for Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) established in 2001 by the Ministry of Agriculture, Livestock and Supply (MAPA), which provides as a way to control through brucellosis vaccination of cattle and buffalo females aged 3 to 8 months, and disposal of animal serum reactants. The vaccine indicated by the program throughout the federation is the B19 sample, whereas the RB51 sample is given to female bovine over eight months ago with negative serology however is not recommended to be use buffaloes (BRAZIL, 2006).

As a zoonotic disease, Brucellosis is caused by gram negative bacteria of the genus *Brucella* (FAGIOLO et al. 2005). For FOSTER et al. (2007) and POESTER et al. (2009) the principal etiologic *Brucella* species which affects in cattle and buffalo is the *Brucella abortus* species (CAPORALE et al. 2010). In spite of this, the genus *Brucella* is described as having other nine species: *Brucella abortus, B. melitensis, B. suis, B. canis, B. ovis, B. neotomae, B. microti, B. ceti* and *B. pinnipedialis* (FOSTER et al. 2007) and have antigenic classification in smooth and rough characteristic.

The diagnosis of brucellosis can be done directly and or indirectly. Within the program currently in effect in Brazil, the serological tests are aggregated as screening tests and confirmatory tests. Are considered screening tests Modified Rose Bengal Plate Test (MRBPT) and Milk Ring Test (TAL); confirmatory tests in the case of animals positive in the screening test are 2 - Mercaptoethanol (2ME), the serum agglutination test Slow (SAL) and Complement-Fixation (FC) (BRAZIL, 2006). Thus, the present study aimed to evaluate the humoral immune response of a group buffalo calves (*Bubalus bubalis*) vaccinated with strain B19 of *B. abortus*, as well as a group that do not has been submit to reagents.

MATERIAL AND METHODS

It was used buffalos 42 animals divided into two groups: the test group included 36 females and the control group formed by six males. The animals

were kept in extensive system in the area of floodplain and mainland mesoregion the lower Amazon, micro region of Santarém, and received identification with earrings and hot iron to prevent miss the plot.

In the selection was took into account the age of three to eight months, as recommended by the program, and before vaccination with B19 sample B. abortus in the standard dose, blood samples was collected from all animals on day 0 , followed by sequential collections every 30 days up to 390 days.

In addition, all females received vaccines against brucellosis according to the manufacturer's instructions (Bovine Vaccine Anabortina B -19, Merial Laboratory®, Paulinia/SP, Brazil) at a dose of 2.0 mL per animal, subcutaneous vaccine Anabortina Bovine B -19 with the use of disposable 3 mL syringe, with needle 25 x 7mm. After this procedure the heifers were marked hot iron on the left side of the face with the letter V, accompanied the final digit of the year of vaccination (V2) as guides PNCEBT the Ministry of Agriculture (BRAZIL, 2001).

However the blood samples collection were performed monthly in the jugular vein using 10 ml syringes and needles 40 x 12 disposable mm and then transfer the bloods to siliconized tubes of 10 ml, and the centrifuged samples for obtaining blood serum were frozen at -20 ° C, and then were transferred to and processed in the Laboratory of Immunology and Microbiology, Institute of Animal Health and Production - ISPA, the Federal Rural University of Amazonia - UFRA in Belém, Pará.

The serum samples were tested in serological tests Modified Rose Bengal Plate Test (MRBPT) and 2-Mercaptoethanol (2-ME) classified as a screening test and confirmatory respectively according Brazil (2006).

RESULTS AND DISCUSSION

All animals in this study were presented in the sample of non-reactive zero day before the vaccination, these results are similar to those found in cattle by POESTER & RECKZIEGEL (1998) and FARIA (2010).

The results obtained throughout the 390-day study, with the humoral response of the heifers are described in Table 1.

Table 1 - Percentage of female buffalo MRBPT tests and reagents to 2-ME after vaccination with *Brucella abortus* strain B19.

Days	MRBPT		2-ME	
	Reagent	%	Reagent	%
0	0	0	0	0
30	36	100	36	100
60	36	100	36	100
90	34	94	36	100
120	23	64	36	100
150	16	44	28	78
180	16	44	17	47
210	14	39	14	39
330	1	3	1	3
360	1	3	1	3
390	0	0	0	0

The percentage of female buffalo reagents for MRBPT evidence and 2-ME were similar at 30 and 60 days, where highest titers were observed in test 2-ME, and 90 days in the early fall of antibodies as shown in Table 2.

Table 2 - Reactive and nonreactive samples MRBPT testing in 2-ME and 36 females vaccinated with B19 strain of *B. abortus* day zero, after 390 days after vaccination.

	MRBPT Sorology		2-ME Sorology		Dilution			
Days	REAG.		REAG.		1:25	1:50	1:100	1:200
	F	%	F	%	F	F	F	F
0	0	0	0	0	0	0	0	0
30	36	100	36	100	36	36	36	36
60	36	100	36	100	36	36	36	36
90	34	94	36	100	0	14	0	22
120	23	64	36	100	0	22	0	14
150	16	44	28	78	7	18	0	3
180	16	44	17	47	7	7	0	3
210	14	39	14	39	4	7	0	3
330	1	3	1	3	0	0	1	0
360	1	3	1	3	1	0	0	0
390	0	0	0	0	0	0	0	0

The males in the control group, were nonreactive for the tests performed during the study period.

Results of the immune response obtained in the present study demonstrated that the use of B19 in female buffalo vaccine is effective which are in accordance with same result obtained by SILVA (2006) that evaluated female buffalo in the recommended age PNCEBT/MAPA and got confirmation on the effectiveness of vaccination after performing the MRBPT exams and 2 - ME in cattle. Also CONCEIÇÃO et al. (2005) confirmed the efficacy of the vaccine B19 through MRBPT proof, but evidence suggested the use of 2 - ME and CF as confirmatory, since there were no cross-reactions and be very specific.

At 30 days after vaccination, 100 % of the animals showed up in serology reagents, starting the decline at 90 days in tests MRBPT and 2 - ME, corroborating the results obtained for cattle by RIBEIRO et al. (1997) and KOLODA (2005) and in buffalo by SILVA (2006) and MUNIR (2009). POESTER & RECKZIEGEL (1998) evaluated the B19 and RB51 vaccine in buffaloes and found a peak in antibody response occurred at 30 days for the two vaccines at 90 days and the beginning of the decline of antigen-antibody reaction.

Some authors reported the occurrence of early fall off vaccine at day 90 titles, which seems to be in agreement with the present study, these experiments also evaluated on their sensitivity and specificity of MRBPT used as screening test and obtained percentages of 91.42 and 94.0 against 95.3 and 91.8 respectively (MOLNÁR et al. 2002; PAULIN et al. 2006).

JAMAL et al. (2003) monitored the immune response of guinea pigs and calves buffalo vaccine prepared from the vaccine strain B19 with the weekly and animals were observed to have a response prepared, but the drop in bond challenged calves was very rapid because at 91 days they had no more vaccine titles as the fall was quite early. Indeed several factors seems to be involved in this phenomenon that may be intrinsic to the preparation of the vaccine and / or extrinsic, either this precocity led the authors to question the safety and efficacy of the vaccine prepared with strain B19 suggesting further studies on the subject.

Regarding the fall of titles in the tests evaluated in the present study, the results differ as to the time obtained by SILVA (2006) POESTER & RECKZIEGEL (1998), DOMINGUES et al. (1992), RIBEIRO et al. (2001), RIBEIRO et al. (1997), where the immunized females showed reagents animals throughout 180 days (six months), 270 days (nine months) 240 days (eight months) and 300 days (ten months), respectively, whereas in the present study time that females reagents remained after vaccination was 360 days (12 months).

POESTER & RECKZIEGEL (1998) described the enduring reaction was due to the presence of animals in the experiment above the age of three to eight months, which differs from the present study although an animal remained reagent for 12 months, however at the date vaccination was below eight months, four months specifically, when taken into consideration the maximum age of the animals were eight month. Furthermore all animals in the study had more than 21 months at the end of blood sample collections, through all within the period provided by the program that 24 months is the time required to re-test the vaccinated animals.

Although it cannot be said that the weather the falloff post vaccinal titles in buffaloes is higher than that of cattle as described by DOMINGUES et al. (1992), more research is needed on the behavior of buffaloes subjected to

vaccination as determined by PNCEBT, rather one can in far reduction, and maintenance increase of time to test vaccinated animals.

On the other hand MUNIR (2009) reported an early fall of vaccine titers in buffalo calves compared to heifers and adult animals, but also reports that a small group of calves and heifers kept their animals vaccinal reagents for 360 days, using the B19 vaccine, coinciding with what occurred in the present study where only one animal maintained the post-vaccination titer within this period (12 months). After 390 days, all animals in the present study had a negative reaction to the MRBPT and 2-ME tests.

CONCLUSION

The buffalo calves vaccinated from three to eight months through the strain B19 vaccine against *Brucella abortus*, was effective and the MRBPT and 2-ME tests, as demonstrated by the positive and negative reactions over 390 days, when all animals were negative.

The expected time of 24 months is recommended tore-test vaccine females from three to eight months according to PNCEBT was confirmed, since the end of the study all animals had maximum age of 21 months when considered maximum age for vaccination was eight months, so within the period specified by the program.

Vaccination cover age of buffalo calves against B. abortus with B19 sample showed to be efficient, however it is essential to seek further scientific information and clarification on the effective ness of the vaccination in this species.

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